Sustained Ocular Delivery of Ciprofloxacin Using Nanospheres and Conventional Contact Lens Materials

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Purpose. To formulate conventional contact lenses that incorporate nanosphere-encapsulated antibiotic and demonstrate that the lenses provide for sustained antibacterial activity.

Methods. A copolymer composed of pullulan and polycaprolactone (PCL) was used to synthesize core-shell nanospheres that encapsulated ciprofloxacin. Bactericidal activity of the nanosphere-encapsulated ciprofloxacin (nanosphere/cipro) was tested by using liquid cultures of either *Staphylococcus aureus* or *Pseudomonas aeruginosa*. Nanosphere/cipro was then incorporated into HEMA-based contact lenses that were tested for growth inhibition of *S. aureus* or *P. aeruginosa* in liquid cultures inoculated daily with fresh bacteria. Lens designs included thin or thick lenses incorporating nanosphere/cipro and ciprofloxacin-HCl-soaked Acuvue lenses (Acuvue; Johnson & Johnson Vision Care, Inc., Jacksonville, FL).

Results. Less than 2 μg/mL of nanosphere/cipro effectively inhibited the proliferation of cultures inoculated with 10^7 or 10^8 bacteria/mL of *S. aureus* and *P. aeruginosa*, respectively. HEMA-based contact lenses polymerized with nanosphere/cipro were transparent, effectively inhibited the proliferation of greater than 10^7/mL of bacteria added daily over 3 days of culture, and killed up to 5×10^9 total microbes in a single inoculation. A thicker lens design provided additional inhibition of bacterial growth for up to 96 hours.

Conclusions. Core-shell nanospheres loaded with an antibiotic can be incorporated into a conventional, transparent contact lens and provide for sustained and effective bactericidal activity and thereby provide a new drug delivery platform for widespread use in treating ocular disorders. (*Invest Ophthalmol Vis Sci.* 2012;53:1341–1352) DOI:10.1167/iovs.11-8215

The treatment of ocular disorders presents a unique challenge for drug delivery systems, since the avascular cornea and blood–aqueous barrier render the eye virtually inaccessible to systemically delivered therapeutics. The most commonly used method of treating acute eye disorders, including bacterial infections, inflammation due to allergic responses and surface injury, and chronic disorders such as glaucoma, is frequent administration of topical ophthalmic solutions in the form of eye drops. However, the challenge of such treatments is to sustain sufficiently high concentrations of the drug in the afflicted tissue.[1,2] Ophthalmic therapeutic solutions are formulated to provide pulse-type drug treatments, since the duration of contact with the corneal surface is very short (1 – 2 minutes), and only a small percentage of the drug (~5%) actually penetrates the cornea. Most of the drug is rapidly lost due to reflex tearing and blinking, spillage during administration, and systemic absorption. High concentrations of drug are therefore required in each drop to drive a useful amount of drug into the eye in a short time. During repeated administration, a significant portion of the applied solution can be absorbed in the conjunctiva or collect in the nasolacrimal system, which drains into the nasal cavity and leads to absorption in the bloodstream. Consequently, systemic side effects can be problematic for many classes of ophthalmic drugs. For example, fluoroquinolones can cause not only corneal but also systemic toxicity (e.g., hepatotoxicity, nephrotoxicity, and neurotoxicity).[3–9] Treatments for glaucoma, in particular β-adrenergic receptor blockers (e.g., timolol), can also produce serious side effects when absorbed into the bloodstream, including cardiac arrhythmias, bronchospasms, depression, and heart failure.[10,11] In addition, the actual dose effectively administered can vary by application technique, the type of eye drop carrier, and compliance of the patient.[12–15] The end result is that during eye drop treatment, the eye is exposed to a series of peak concentrations that are potentially toxic, followed by inadequate concentrations until the next dose. Furthermore, because the cornea and other ocular barriers such as the sclera typically absorb only a small percentage of drug, doses must be frequently administered; in severe cases such as acute bacterial keratitis, dosing is required as often as every 15 to 30 minutes.[16,17] Improved topical solutions with increased viscosity or added mucoadhesive solutions increase retention time of drug on the cornea, but can be accompanied by irritation, difficulties in application and reduced vision, and may not provide for sustained (e.g., greater than 1 to 2 hours) drug delivery.[18–21] The complications and inefficiencies associated with topical administration of drugs to the eye have prompted the pursuit of alternative approaches that provide prolonged and sustained drug delivery, minimal toxicity, and ease of use.

A well-studied and convenient method of providing sustained drug delivery is the use of specially formulated soft hydrogel contact lenses; by design, such contact lenses already provide comfort, clarity, and biocompatibility, and therefore are an attractive choice for ocular drug applications. Early studies reported the use of hydrogels for ocular drug delivery using commercial contact lenses that were soaked in various antibiotics and/or ocular therapeutics for 2 to 4 hours and then rinsed for different times and monitored for drug release.[22,23] These drug-soaked lenses successfully provided for longer periods of drug application compared with eye drops, but nev-

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ertheless most drugs rapidly dispersed from the lenses within hours. For example, the corticosteroid prednisolone, the glaucoma drug pilocarpine, and the antibiotic ciprofloxacin were shown to be effectively absorbed by several different types of soaked contact lenses, but nearly all of the drug release occurred within 1 to 3 hours.[23–26] Levels of drug uptake versus release were variable and depended on the material chemistry of the contact lens (e.g., silicon-based versus conventional, or ionic versus nonionic). Distribution of some drugs, including ciprofloxacin, can be inconsistent due to drug precipitation at the lens surface.[27] Furthermore, a critical limitation to sustained release rates is the inherently high surface area-to-volume ratio of a standard contact lens design.[27,28] Thus, soaked lenses provide an initial burst of drug release that is short lived, and the lenses may become ineffective in less than 24 hours after application.

Several modifications have recently been incorporated into lens synthesis to provide a reservoir of drug within the lens that is slowly released in a sustained fashion. Modifications include imprinted hydrogels in which lens polymer synthesis is designed to create functional monomers that enhance drug uptake and that increase the duration of drug delivery.[29,30] Such formulations increase the drug delivery times beyond that achieved by eye drops and do not require presoaking in the drug or special rinsing by the user. For example, release of the antihistamine ketotifen fumarate was shown to occur over an 8-hour period, whereas a second design extended the release of norfloxacin to 3.5 days.[31,32] Biomimetic hydrogels have also been generated with functional monomers that mimic the noncovalent interactions of drugs such as the antihistamine ketotifen, and these lenses extended release times up to 5 days.[33] Recent approaches have also used novel drug-bearing microemulsion droplets, or liposomes, incorporated within conventional contact lens materials, most commonly poly-hydroxyethyl methacrylate (p-HEMA). Liposomes loaded with therapeutics have also been surface applied in layers by means of immobilizing agents such as polyethylenimine (PEI) or a co-polymer layered system composed of poly(lactic-co-glycolic) acid (PLGA) and pHEMA.[32,34–38] These studies have demonstrated improvements in the sustained release of multiple drugs, including lidocaine, timolol, and two fluoroquinolones, levofloxacin, and ciprofloxacin. Together, these studies indicate that sustained drug release can be achieved with these sophisticated systems, in some cases for up to 2 weeks.[37] However, limitations of such approaches include the added complications of specially engineered lens designs, such as lens molds that provide separate reservoirs of drug delivery formulas or successive casting steps required for applying layers of drug-loaded liposomes, a lack of lens clarity (at least in the region of drug incorporation), and/or a bolus of drug release over the first few hours followed by drug exhaustion within 24 hours.[36–38] Few of these studies analyzed antibacterial activity or the efficacy of the released drug, and effects of the modified lenses on bacterial growth in at least one study were inferior to that provided by drug-soaked lenses.[36]

Nanospheres that encapsulate drugs are a relatively new and attractive drug delivery system that provides for localized drug delivery and sustained drug release.[39] They can be functionalized to encapsulate both hydrophilic and hydrophobic forms of therapeutics and shield the active agents from harsh external environments.[40] Studies have investigated the capacity of nanospheres composed of PLGA to encapsulate and release hydrophilic ciprofloxacin HCl, but analyses of antibacterial activity of the particles showed lower efficacy than did hydrophobic free ciprofloxacin, and these studies did not use nanospheres amenable to hydrophobic drugs.[41,42] By comparison, core-shell nanospheres are unique in that their amphiphilic structure provides for improved water solubility of hydrophobic drugs.[40,43–45] We recently described the characteristics of self-assembling core-shell nanospheres composed of a copolymer of an hydrophobic polyester, polycaprolactone (PCL), plus an hydrophilic polysaccharide that is nontoxic and non-immunogenically biodegradable (pullulan).[46] When synthesized to encapsulate therapeutics such as antibiotics, these pullulan-PCL core-shell nanospheres create a reservoir of drug that is slowly released and therefore amenable for sustained drug delivery. To test this application, pullulan-PCL core-shell nanospheres were used to encapsulate the hydrophobic (free-base) form of ciprofloxacin, chosen due to its broad spectrum of antibacterial activity against both Gram-positive and -negative bacteria and because ciprofloxacin is commonly prescribed for treating keratitis caused by two of the most clinically significant forms of ocular bacteria, *S. aureus* and *P. aeruginosa*.[47,48] Furthermore, resistance to ciprofloxacin is known to develop slowly, and the drug is well-tolerated.[3,38,49] Our studies showed that release of ciprofloxacin from the nanospheres primarily occurs over the first 3 days of analysis, but continues for an additional 7 days.[46] Here, we describe the synthesis of conventional hydrogel contact lenses that incorporate nanosphere-encapsulated ciprofloxacin (nanosphere/cipro), and demonstrate that the released ciprofloxacin from two different types of lenses continuously inhibits the growth of either *S. aureus* or *P. aeruginosa* in liquid cultures. Of note, serial inoculations with fresh bacteria into liquid cultures containing the lenses demonstrated sustained antibacterial activity over several days of culture. The antibacterial activity of each lens type was compared to that provided by ciprofloxacin-soaked Acuvue lenses (Johnson & Johnson Vision Care, Inc.).

Materials and Methods

Synthesis of conventional HEMA lenses was performed with the following components: the monomer of >99% 2-hydroxyethyl methacrylate (HEMA), inhibited with 50 ppm mono-methylether of hydroquinone (MEHQ); the cross-linker poly(ethylene glycol) dimethacrylate (PEGDM), inhibited with 250 ppm MEHQ; the initiators 2, 2-azobis(2-methylpropionamidine)dihydrochloride (azo HCl), and the oligo [2-hydroxy-2-methyl-1[4-(1-methylvinyl) phenyl] propanone] 2-hydroxy-2-methyl-1-phenyl propan-1-one (monomeric; Esacure KIP 100F; Lamberti SpA, Chattanooga, TN). All monomers and 1-methyl-2-pyrrolidinone were obtained from Sigma-Aldrich (St. Louis, MO). Free-base ciprofloxacin and ciprofloxacin-HCl, a monohydrochloride salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid, were purchased from LKT Laboratories (St. Paul, MN). An ophthalmic, preservative-free formulation of ciprofloxacin 0.3% was used for soaking Acuvue lenses, which was obtained as a ready-to-use solution from Lieter's Pharmacy (San Jose, CA). Bacterial strains of *S. aureus* and *P. aeruginosa* were obtained from the American Type Culture Collection (ATCC, Manassas, VA; 6538 and 9027, respectively). Bacteria were cultured in trypticase soy broth (TSB) or on trypticase soy agar (TSA) plates (BD Biosciences, Franklin Lakes, NJ).

Synthesis of Nanosphere-Encapsulated Ciprofloxacin

Synthesis of the pullulan-polycaprolactone nanoparticles was performed as previously described.[46] Briefly, nonhydrolyzed pullulan was dissolved in deionized water at 90 mg/mL, to which hydrochloric acid (0.1 M) was added, and the mixture was incubated at 60°C for 40 hours. polycaprolactone (PCL) was dissolved in 1-methyl-2-pyrrolidone (NMP) with pyridine, nitrophenyl chloroformate was added, and the mixture of PCL nitrophenyl carbonate was stirred for 1 hour. Both solutions were precipitated with methanol and dried under a vacuum. Next, hydrolyzed pullulan was dissolved in NMP, 4-dimethylamino pyridine was added with the PCL nitrophenyl carbonate, the mixture was stirred for 48 hours, and the solution was precipitated with

ethanol. After a toluene wash, the solid residue was dried for 24 hours under vacuum.

To prepare the nanospheres, 100 mg of block copolymer was dissolved in 2 mL of dimethyl sulfoxide (DMSO), along with 15 mg of the free-base ciprofloxacin. Deionized water (10 mL) was then added drop-wise during constant stirring to form the micelles. To remove the DMSO, the solution was placed in dialysis cassettes and submerged in water that contained 1.8 mg/mL ciprofloxacin-HCl, as described elsewhere.[46] After 48 hours of dialysis, the nanosphere solution was removed, frozen, and placed in a freeze dryer.

Atomic Force Microscopy

A photograph of representative drug-free pullulan-polycaprolactone nanospheres was taken by dispersing the nanospheres in water to form a colloidal solution, which was dried on mica slides in a desiccator on a Petri dish. An area of 2 μm^2 was scanned in contact mode with an applied force of ~10 nN using a probe head scanner with an Si_3N_4 tip (Autoprobe CP-Research; ThermoMicroscopes, Sunnyvale, CA).

Synthesis of Hydrogel Lenses Incorporating Nanosphere/Cipro

To create contact lenses incorporating nanosphere/cipro, two formulations were used that resulted in different lenses: an initial formulation that yielded a thinner lens and a second formulation that generated a thicker lens with additional incorporated nanosphere/cipro. The thin lens was generated by vortexing a mixture of 400 mg of nanosphere/cipro in 2 mL of H_2O, which was then added to 3 mL of a solution composed of 99% HEMA, 1% PEGDM, and 50 mg/mL photoinitiator (KIP 100F; Lamberti, SpA). The mixture was vortexed to create a cloudy suspension, and the precipitate was allowed to settle for 60 minutes at room temperature. The supernatant was then filtered through a 5-μm syringe, and a sufficient amount of the slightly hazy eluent was added to fill the bottom polypropylene contact lens casting cups. After the tops were placed on the cups to form sealed molds, they were treated in a UV oven (UVPS CL-1000L) at 365 nm for 30 minutes (this is a dose range of ~268,000 $\mu J/cm^2$/min, for a total dosage of ~8 J/cm^2). After opening the molds, the lenses were freed by placing the mold half with the lens in a solution of 0.5% ciprofloxacin-HCl for 10 to 15 minutes to minimize possible loss of ciprofloxacin from within the lenses. This process yielded lenses with weights of 30 ± 4 mg and approximately 10 mm in diameter. The thicker lenses were generated by vortexing a 1-mL solution of 7.5 mg/mL Azo HCl with 150 mg of the nanosphere/cipro mixture to form a dispersed solution. Then 1.5 mL of HEMA was added to this solution, the mixture was vortexed and passed through a 5-μm filter, and the resulting hazy solution was used to fill bottom casting cups (proprietary design, Vista Scientific, Andover, MA). The top and bottom molds were then sealed and placed in a 50°C oven for 3 days. The polymerized lenses that weighed approximately 66 mg were then removed and soaked in PBS before drug release assays and addition to liquid bacterial cultures.

Bacterial Culture

The proliferation of the bacterial cultures was determined by monitoring changes in turbidity using absorbance measurements at 600 nm with a microplate reader (Spectramax M2; Molecular Devices, Sunnyvale, CA). The changes in absorbances were then converted to corresponding colony forming units (CFU) per mL using a titration curve. The titration curve was generated by taking samples of bacteria at OD_{600} of 0.05 to 2.0, serially diluting the cells in TSB and then culturing the diluted cells on TSA plates overnight at 37°C. Colonies were counted, and data from triplicate assays were used to generate a titration curve that compared OD_{600} measurements with colony forming units per milliliter for the growth of both bacterial strains (data not shown).

Inhibition of *S. aureus* and *P. aeruginosa* by Ciprofloxacin and Nanosphere/Cipro

To determine the minimum concentration of ciprofloxacin-HCl effective in inhibiting the growth of *S. aureus* and *P. aeruginosa*, concentrations of drug ranging from 0.1 to 2.5 μg/mL were added to 5 mL of TSB, which was inoculated with 5 μL of fresh bacteria from overnight cultures, to yield final concentrations of approximately 1×10^7 CFU/mL for *S. aureus* or 1×10^8 CFU/mL for *P. aeruginosa*, each based on OD_{600} measurements after inoculation. Similar assays were performed using 2.5, 5.0, and 7.5 μg/mL of nanosphere-encapsulated drug added to *S. aureus* cultures, or 5, 7.5, and 10.0 μg/mL of nanosphere-encapsulated drug added to *P. aeruginosa* cultures. For all assays, the cultures were incubated at 37°C on a shaking incubator at 225 to 250 rpm and changes in OD_{600} were measured at 2-hour intervals up to 8 hours and then at 24-hour intervals. Corresponding bacteria quantities were then calculated using the titration information as described above. Data shown are reported as the average ± standard deviation of results in triplicate experiments. Control samples, included in each assay, were inoculations in TSB without added drug to demonstrate normal bacteria proliferation (data not shown).

Antimicrobial Activity of Hydrogel Lenses Incorporating Nanosphere/Cipro

The sustained antimicrobial effectiveness of released ciprofloxacin from the two prototype lenses with incorporated nanosphere/cipro was tested with *S. aureus* or *P. aeruginosa* in liquid cultures. Included in each study were Acuvue lenses (Johnson & Johnson Vision Care, Inc.) that were soaked in 0.3% of ciprofloxacin solution for 4 hours and then rinsed in phosphate buffered saline (PBS, containing [in mM] 137 NaCl, 2.7 KCl, 4.3 $Na_2HPO_4 \cdot 7H_2O$, and 1.4 KH_2PO_4 [pH 7.4]) for 15 minutes. For each of the two different lens configurations (thin versus thick) studied, the lenses were rinsed in 5 mL of PBS for 15 minutes and then introduced into 5 mL of TSB that was inoculated with 5 μL of bacteria from fresh cultures (again yielding final concentrations ranging from 10^7 to 10^8 CFU/mL medium). Changes in OD_{600} were measured every 2 hours for the first 8 hours and then at 24 hours. Lenses were then removed from the cultures and placed in 5 mL of fresh medium with a new inoculation of bacteria. The cultures were allowed to grow for another 24 hours, OD_{600} measurements were performed, and then each lens was transferred to fresh medium with a third spike of bacteria; the thin lens experiment ended with the third spike, but the thicker lenses were further analyzed with a fourth spike of bacteria. Changes in CFU/mL corresponding to the OD_{600} measurements were calculated by using the titration curve data. For each inoculation, a control tube was also inoculated, and absorbances were measured during the first 8 hours to show growth of the fresh bacteria. Data are expressed as the average ± standard deviation of results of three independent assays/lenses.

To test the capacity of the nanosphere/cipro-incorporating lenses to inhibit the growth of higher concentrations of exponentially growing bacteria, 5 mL of TSB was inoculated with bacteria to a final concentration of approximately 1×10^7 CFU/mL of *S. aureus*, and the culture was allowed to expand to a density of approximately 1×10^9 CFU/mL, which occurred after 4 hours of culture. The thin lenses were then introduced into tubes of the log-phase growing bacteria and OD_{600} measurements were performed at 4, 8, 24, 32, 36, 48, 52, and 56 hours. After 72 hours of culture, an OD_{600} measurement was taken, and the lenses were transferred to a second culture of fresh bacteria containing approximately 10^9 CFU/mL. OD measurements were then performed at 76, 80, and 96 hours. All assays were performed in a 37°C shaking incubator at 250 rpm. Data are the average ± standard deviation of results of triplicate assays.

Drug Release Studies

Solutions of ciprofloxacin-HCl, in a concentration range of 5 to 1000 ppm, were prepared in buffer (Purilens Ultra PF; The LifeStyle Co.,

Wall Township, NJ). A UV scanning diode array spectrophotometer (model 8452A; Hewlett Packard, Palo Alto, CA) was used to generate a calibration curve of ciprofloxacin-HCl. The wavelength used for all analyses was based on a UV spectral analysis that revealed a peak absorbance at 271 nm. This peak absorbance was identical between samples of pure ciprofloxacin and that released by the nanospheres (data not shown). To conserve nanospheres throughout the assays, water was used to blank each measurement, because samples of water with dispersed drug-free nanospheres showed no change in absorbance at 271 nm. A calibration curve for ciprofloxacin free base was generated by molar conversion from the salt. To test for the amount of encapsulated ciprofloxacin within the nanospheres, freeze-dried samples were diluted in water at different concentrations, and peak absorbances at 271 nm were obtained for each dilution. A standard curve for ciprofloxacin was then used to convert absorbances into weights. For measuring drug release from the nanosphere/cipro-incorporating thin lenses, three lenses weighing approximately 30 mg each were placed in 4-mL glass vials to which 2 mL of buffer was added. After 24 hours at 37°C, each sample was removed and placed in another 4-mL vial and covered with 2 mL of fresh buffer. The 24-hour release vial was capped, labeled, and held for analysis. This procedure was repeated every 24 hours to obtain release data for the first 3 days, and then at 2- to 4-day intervals for a total of 14 days. The drug release samples were analyzed by UV spectroscopy (λ_{max} = 271 nm), and absorbance readings were converted to weight of drug via the calibration curve. A similar procedure was then performed with a thick lens sample weighing approximately 66 mg.

Results

Characterization of Drug Release and Antibacterial Activity Provided by Nanosphere/Cipro

We previously coupled hydrolyzed pullulan with polycaprolactone (PCL) to generate a pullulan-PCL copolymer that could be assembled in an aqueous environment into micelles of uniform size; when dissolved in DMSO with ciprofloxacin free base, this created nanospheres that encapsulated the hydrophobic form of ciprofloxacin (Fig. 1).[46] After synthesis, the nanosphere solution was subjected to dialysis by using ciprofloxacin-containing water (which helped to maintain ciprofloxacin levels in the nanospheres) for 48 hours to remove the DMSO, filtered, frozen, and freeze dried. Our previous analyses of the resulting samples showed that the dried nanospheres obtained a uniformly white, cottonlike appearance that dispersed rapidly in water, yielded a clear solution, and showed no evidence of degradation, at least over 2 to 3 months of handling the dried material.[46] The dispersed ciprofloxacin-containing nanospheres (nanosphere/cipro) were uniform in size with diameters of 142 ± 12 nm (Shady, et al., manuscript submitted). In addition, drug release studies were conducted (as described in the Materials and Methods section) that revealed 10 mg of this initial nanosphere/cipro preparation released 1934 ± 0.02 μg of ciprofloxacin over the first 24 hours, followed by 390 ± 0.11 μg over the second day and then 76 ± 0.09 μg over the third day of analysis. Less than 100 μg was released over an additional 7 days of analysis (several days yielded levels below that detectable by the spectrophotometer), and a total of ~2.5 mg was released from the starting 10 mg of nanosphere/cipro (or ~25% by weight).[46] Based on the success of these studies, we synthesized more batches of nanosphere/cipro that were used throughout the current investigation.

To begin the current investigation, we first wanted to predict the amounts of nanosphere/cipro that would be required to effectively inhibit the growth of *S. aureus* and *P. aeruginosa*, the two most common bacteria encountered in ocular infections. We began by testing different concentrations of

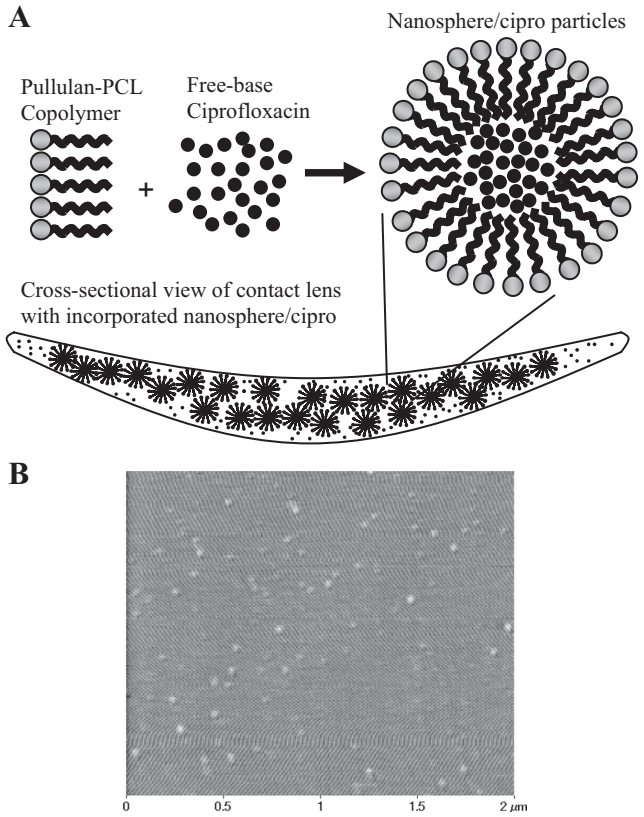

Figure 1. Depiction of nanospheres used to encapsulate ciprofloxacin. (**A**) Depicted are the copolymers that consist of hydrophilic pullulan (*shaded spheres*), linked to a hydrophobic chain of polycaprolactone (PCL, *jagged lines*), that were assembled in the presence of free-base ciprofloxacin (*solid spheres*) to form micelles. The nanospheres were then incorporated into conventional contact lenses. (**B**) Shown is an atomic force microscopy image of empty nanospheres, demonstrating that the pullulan-PCL copolymers assemble into distinct nanometer-sized spheres.

ciprofloxacin for growth inhibitory effects on proliferating liquid cultures of each bacterium. Our preliminary studies of ciprofloxacin added to cultures of the Gram-positive bacteria *S. aureus* showed that quantities equal to or less than 1.2 μg/mL of inoculated broth failed to completely inhibit bacterial growth (data not shown), but subsequent studies showed that 1.5 μg/mL of ciprofloxacin completely inhibited the proliferation of approximately 10^7 CFU/mL for 48 hours (Fig. 2A). Studies performed with *P. aeruginosa* demonstrated that similar amounts of ciprofloxacin inhibited growth of this Gram-negative bacteria for 48 hours (Fig. 2B). Antibacterial activity of the nanosphere/cipro was then tested, using amounts based on our previous study of the initial batch of nanosphere/cipro that demonstrated ~25% by weight of nanosphere/cipro is bioavailable ciprofloxacin. As shown in Figure 3, nanosphere/cipro effectively inhibited the growth of greater than 10^7 CFU/mL of both bacterial strains for up to 48 hours. We note that the titration curves revealed that the amount of *P. aeruginosa* contained in cultures with absorbances at OD_{600}, which was equivalent to that in *S. aureus* cultures, was approximately 10-fold higher, which explains why *P. aeruginosa* cultures consistently yielded a higher number of surviving cells during the assays. The minimum inhibitory concentration (MIC) of ciprofloxacin necessary to inhibit the growth of *P. aeruginosa* is higher than that of *S. aureus* (0.25–1.0 μg/mL and 0.12–0.5 μg/mL, respectively; According to the U.S. Food and Drug Administration's Summary Basis for Approval for Cipro), which

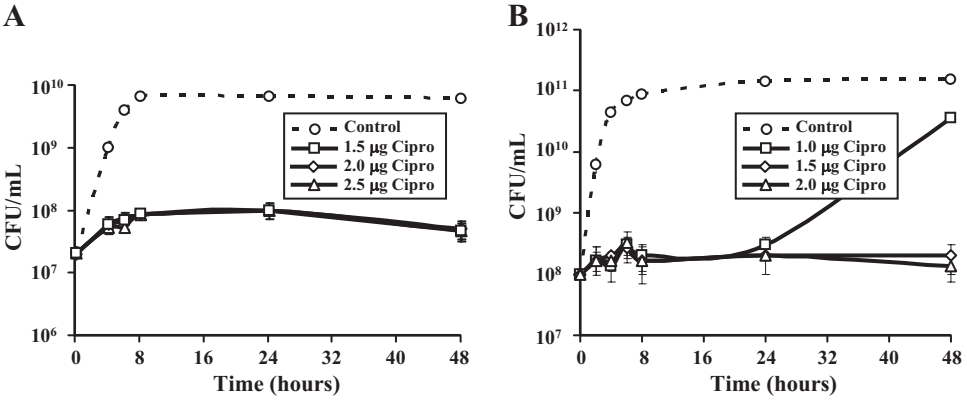

FIGURE 2. Antibacterial activity provided by ciprofloxacin-HCl in liquid bacterial cultures. Different quantities of ciprofloxacin-HCl were added to 5 mL of broth that was inoculated with either (**A**) *S. aureus* or (**B**) *P. aeruginosa* from fresh overnight cultures. Optical densities at 600 nm were then measured and then converted to corresponding colony forming units per milliliter. Control indicates bacterial growth in broth alone.

is consistent with our results that greater amounts of nanosphere/cipro were necessary to completely inhibit *P. aeruginosa* cell growth than was needed to inhibit the growth of *S. aureus* (7.5 vs. 10 μg/mL).

Generation of Hydrogel Lenses Incorporating Nanosphere/Cipro

Two different types of lenses were synthesized, each using different quantities of a mixture of the monomer HEMA, together with newly synthesized batches of nanospheres with encapsulated ciprofloxacin. Both lens formulas yielded conventional hydrogel-type lenses with a typical thin design; the thinner lenses weighed 30 ± 4 mg and were similar to commercial soft contact lenses, whereas the thicker lenses weighed approximately double that of the thin lenses. Both types of lenses were transparent and measured approximately 10 mm in diameter (see Fig. 4A for an example of the thicker lens). Based on the amounts of nanosphere/cipro added to the lens formulas, the thinner lenses were estimated to be composed of 7.41% nanosphere/cipro, or ~2.2 mg nanosphere/cipro in the 30-mg polymerized lenses. The thicker lenses were estimated to contain 5.66% nanosphere/cipro, or approximately 3.7 mg of nanosphere/cipro per lens. We also generated Acuvue lenses (Johnson & Johnson Vision Care, Inc.) loaded with clinical-grade ciprofloxacin by soaking the lenses in the drug for 4 hours and then rinsing them in PBS. We note that the ciprofloxacin used for soaking the lenses was a commercially prepared, preservative-free 0.3% ophthalmic formulation and different from that solution prepared in the laboratory to free the synthesized lenses from their molds; this commercial product was used with the Acuvue lenses so that we could compare how the hydrophobic drug released by the lenses incorporating nanosphere/cipro would compare to a currently available option (i.e., via soaking an Acuvue lens in a commercial ophthalmic ciprofloxacin product available from a pharmacy). Despite the convenience of this process, the soaked lenses were consistently cloudy (Figs. 4B, 4C, untreated lens versus soaked lens, respectively), supporting previous studies that indicated soaking of contact lenses in this drug results in precipitation of the drug at the lens surface.[27] Even after several days of soaking the lenses in PBS, the precipitate remained evident. In contrast, the hydrogel lenses incorporating nanosphere/cipro retained clarity with no further treatments after synthesis.

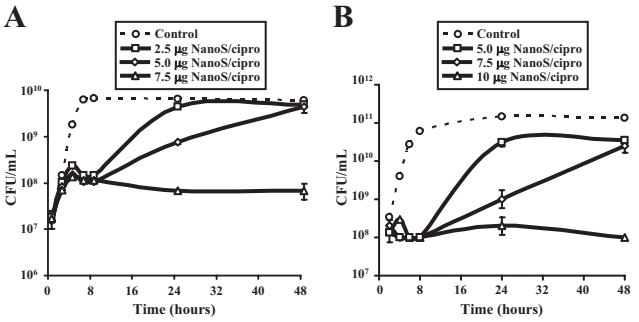

FIGURE 3. Antibacterial activity provided by nanospheres with encapsulated free-base ciprofloxacin. Different quantities of nanosphere/cipro were added to broth that was inoculated with either (**A**) *S. aureus* or (**B**) *P. aeruginosa*, using fresh overnight cultures. The number of colony-forming units corresponding to OD_{600} measurements were then calculated and plotted. The control indicates growth of bacteria in broth alone.

Drug-Release Profiles of Nanosphere/Cipro-Incorporating Lenses

The thinner lenses were tested for ciprofloxacin release over a 14-day period to identify how much drug was released each day in an aqueous environment. For each assay, a typical sized lens (e.g., ~30 mg for the thin lenses and ~66 mg for the thick lens) was placed in PBS, and the released ciprofloxacin was identified by assessing absorbance changes at 271 nm. As shown in Table 1, much of the drug was released during the first 24 hours (~258 μg total), followed by a slower release of approximately 12 and 4 μg total drug per lens during the days 2 and 3, respectively. By day 14, ciprofloxacin release had diminished to approximately 0.2 μg/d per lens. The cumulative amount released over 14 days of analysis by an average thin lens adjusted to a weight of 30 mg was 279 ± 21 μg. This amount is less than predicted by the estimated total amount of nanosphere/cipro incorporated in each lens (e.g., 2.2 mg) and assumes that ~25% of this weight is available ciprofloxacin (as suggested by drug release studies on the initial batch of nanosphere/cipro).[46] By comparison, a preliminary study of a thick lens showed that ~900 μg of ciprofloxacin was released during the first 24 hours, followed by ~250 μg during the second day, and 26 μg during the third day, with a cumulative amount of ~1.2 mg after 14 days of release. Based on the estimated amount of nanosphere/cipro incorporated into this thicker lens (3.7 mg), these data indicate that greater than 30% of the nanosphere/cipro used to synthesize this type of lens is available ciprofloxacin. Since different batches of nanosphere/cipro were used to synthesize each type of lens, together these studies suggest that batches of nanosphere/cipro contain variable amounts of ciprofloxacin that range from 15% to 30% by weight. The different procedures used to synthesize each type of lens may also affect the total amount of nanosphere/cipro that actually becomes incorporated into each type of lens. Nonetheless, the amounts of ciprofloxacin released over the first 48 hours by either lens far exceeds the known MIC values

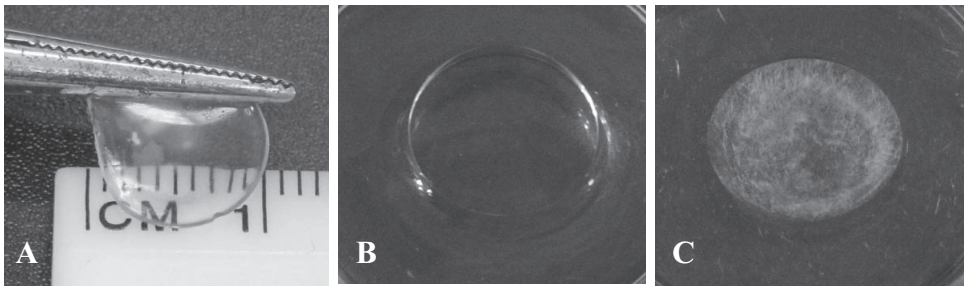

FIGURE 4. Properties of the nanosphere/cipro-incorporating hydrogel lenses compared to ciprofloxacin-soaked lenses. (**A**) Photograph of the polymerized HEMA lens with nanospheres illustrates transparency and size. Photographs of Acuvue lenses (**B**) before and (**C**) after soaking in ciprofloxacin 0.3% demonstrates drug precipitation.

for ciprofloxacin to inhibit the growth of either *S. aureus* or *P. aeruginosa*, and therefore each lens should continuously inhibit the growth of either bacterium in liquid cultures.

Our next series of assays tested this possibility by transferring the lenses to continuous cultures and analyzing bacterial growth after multiple inoculations.

Antibacterial Activity of Nanosphere/Cipro-Incorporating Lenses in Broth Cultures

The duration of antibacterial activity provided by the ciprofloxacin that was released by the thin nanosphere/cipro-incorporating lenses was demonstrated using a series of liquid cultures inoculated with live bacteria and then incubated with the lenses. Acuvue lenses presoaked in ciprofloxacin were also tested, and cultures in the absence of added lenses served as the negative controls. As shown in Figure 5, both types of lenses completely inhibited growth of both bacterial strains during the first 8 hours and up to 24 hours, whereas medium alone showed log-phase growth within 2 hours. After transfer to fresh broth inoculated with live bacteria, both lenses continued to inhibit bacterial growth for another 24 hours (for a total of 48 hours). Furthermore, medium taken from these cultures after incubation with the lenses for each 24-hour period failed to exhibit bacterial growth, indicating that the released ciprofloxacin not only inhibited any further growth, but also killed all live bacteria within the cultures. A third transfer was then performed into broth inoculated with fresh bacteria, and growth was now observed in medium with the nanosphere/cipro-incorporating hydrogels at 52, 56, and 72 hours. By comparison, the ciprofloxacin-soaked Acuvue lenses exhibited inhibition up to 72 hours, when growth was just starting to occur. We also tested lenses that incorporated drug-free nanospheres against both *S. aureus* and *P. aeruginosa* cultures to demonstrate that the nanospheres themselves had no effect on bacterial growth; as expected, these control lenses did not inhibit bacterial growth (Fig. 6). Finally, the control lenses with incorporated drug-free nanospheres were also soaked in ciprofloxacin as previously performed with the Acuvue lenses. Although these drug-soaked lenses inhibited bacterial growth for the first 24 hours, they failed to inhibit growth during the next 24 hours, whereas the Acuvue cipro-soaked lenses provided antibacterial activity beyond 48 hours. Together these data confirm that the only antibacterial activity provided by the nanosphere/cipro-incorporating lenses is that caused by release of the encapsulated ciprofloxacin, and although inhibition of bacterial growth was not quite as prolonged as that provided by the Acuvue/cipro-soaked lenses (48 vs. 72 hours), the clear nature of the nanosphere/cipro-incorporating lenses make them a better choice for clinical applications.

TABLE 1. Comparison of Ciprofloxacin Released from Nanosphere/Cipro-Incorporated Lenses

	Thin Lens (n = 3)		
Days	Avg (μg)	SEM	Thick Lens Avg (μg)
1	255.938	5.152	927.954
2	11.709	0.966	258.431
3	4.119	0.061	26.896
5	1.499	0.106	2.935
7	1.034	0.108	0.660
10	0.393	0.018	0.214
14	0.206	0.026	0.00
Total amount released/lens	274.90		1217.09

All amounts are in micrograms released per hydrogel.

The next assay tested antibacterial activity of the thin nanosphere/cipro-incorporating hydrogel lenses in cultures that were allowed to reach log phase growth such that the culture contained approximately 1×10^9 CFU/mL, which was performed before addition to the lenses. As shown in Figure 7, in the absence of lenses the bacterial concentration continued to increase to the stationary phase, but growth rapidly diminished on addition of the lenses, with complete arrest occurring by 8 hours. Thus, both the nanosphere/cipro-incorporating lenses and ciprofloxacin-soaked Acuvue lenses effectively inhibited further proliferation of high concentrations of bacteria for up to 56 hours (Fig. 7, left). As previously performed, samples of the inoculated medium after lens treatment were tested for live bacteria, but no growth was observed, indicating that the released ciprofloxacin killed all bacteria in the cultures (the observed optical densities are most likely caused by dead bacteria and debris). At 72 hours, the lenses were transferred to a second fresh culture of log-phase growing bacteria (again, $\sim 1 \times 10^9$ CFU/mL), and OD_{600} measurements were performed at 76, 80, and 96 hours (Fig. 7, right). Although bacteria grew in medium with either type of lens, the total amount of bacteria at any time point during this analyses was significantly less in medium with lenses compared to the control medium (e.g., for the 80 hour time point, differences between nanosphere/cipro-incorporating lenses versus the positive control were significant with $P < 0.0001$, as determined by Student's t-test with equal variances). Together these data demonstrate that the nanosphere/cipro-incorporating thin lenses were efficient at inhibiting the growth of 5×10^9 total bacteria (the amount contained in 5 mL of medium), most if not all of which were dead by 56 hours, and there was enough residual ciprofloxacin to at least inhibit the growth of the additional bacteria added after 72 hours.

In the final analyses, the thicker lenses were tested for their capacity to inhibit bacterial growth using assays identical with those described above. As shown in Figure 8A, the thicker nanosphere/cipro-incorporating lenses inhibited *S. aureus* growth during the first 24 hours and then continued to inhibit growth after three additional transfers into cultures of fresh bacteria, each cultured for an additional 24 hours, for a total of 96 hours. The lenses were then transferred a fourth time after 96 hours into inoculated medium, but both the nanosphere/cipro-incorporating lenses and the ciprofloxacin-soaked Acuvue lenses showed bacterial growth near that of the negative control, and completely lacked antibacterial activity after the

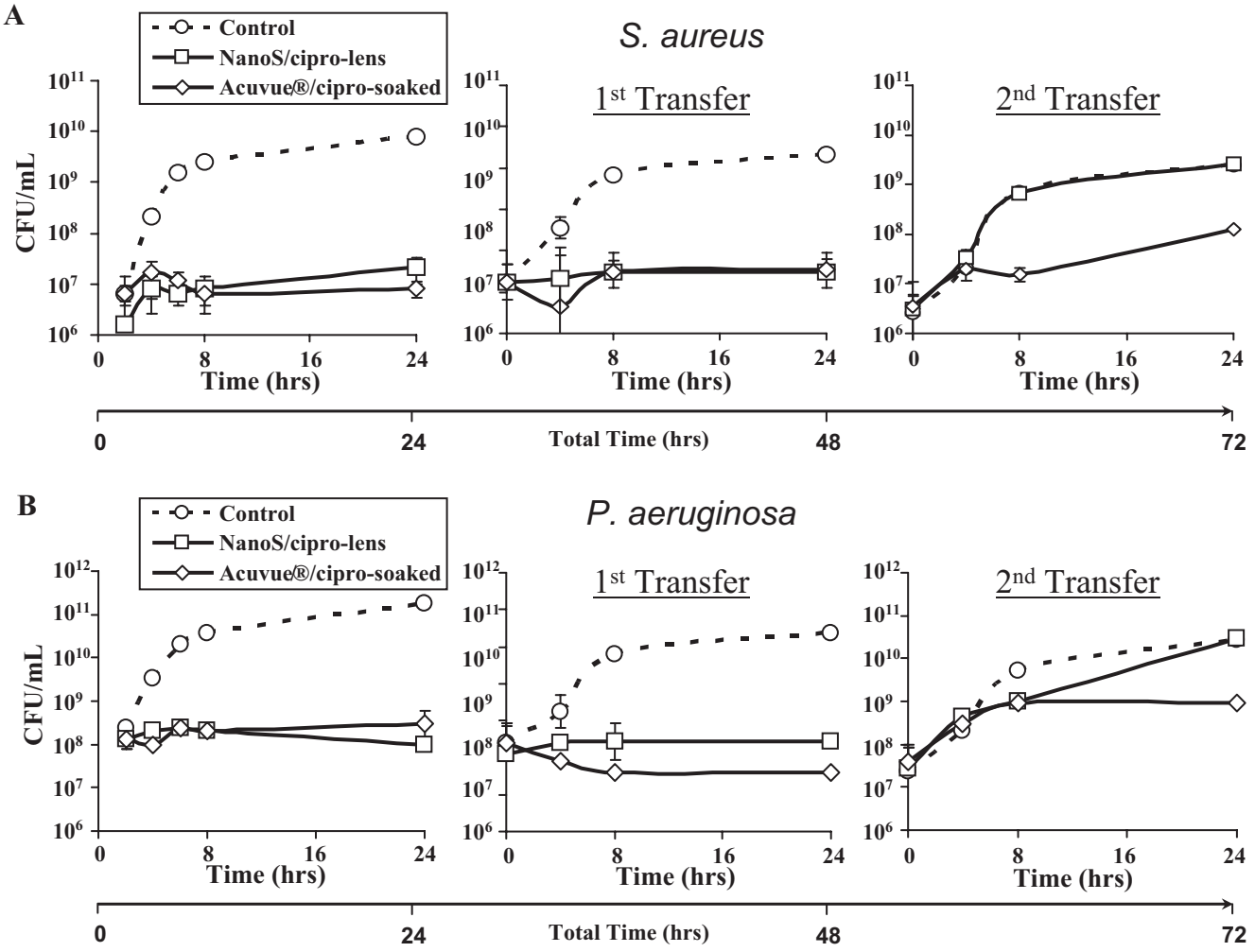

Figure 5. Antibacterial activity of nanosphere/cipro-incorporating thin lenses. Two types of lenses were generated: one set synthesized with nanosphere/cipro (NanoS/cipro-lens) and a second set of Acuvue (Johnson & Johnson Vision Care, Inc.) lenses soaked in ciprofloxacin (Acuvue/cipro-soaked), each of which were added to liquid bacterial cultures inoculated with (**A**) *S. aureus* or (**B**) *P. aeruginosa*. Colony-forming units per milliliter were determined from OD_{600} readings taken at 2, 4, 6, and 8 hours after inoculation and then again at 24 hours (*left*). The lenses were then transferred to fresh cultures reinoculated with fresh bacteria, along with tubes with broth alone for controls, and CFU amounts were determined at the indicated times (*middle* and *right*). Shown below each graph set is a time line indicating the total time of each experiment.

fifth transfer. The nanosphere/cipro-incorporating thick lenses also effectively inhibited the growth of *P. aeruginosa* after two transfers and a total of 72 hours (Fig. 8B). Bacterial growth was then observed shortly after the third transfer was performed at 72 hours. The ciprofloxacin-soaked Acuvue lenses exhibited similar antibacterial activity against *P. aeruginosa*, but they appeared to inhibit some growth after the third transfer, which lasted for at least 8 hours of incubation. Together these studies indicate that different lens designs can be formulated to adjust the amount of drug incorporated into the lens, which will provide for different drug release profiles and maintain different antibacterial activities after application.

Discussion

When considering the design of a new drug delivery system for treating ocular infections, three factors must be considered: The type of antibiotic that will be incorporated for release from the system must be applicable to an appropriate range of infectious agents, the material used to encapsulate the drug must be functionalized to reversibly trap the drug and provide for sustained release, and the delivery mechanism must be applicable to ophthalmic applications and suited to large-scale manufacture. With these factors in mind, we synthesized hydrogel contact lenses using a conventional formulation (HEMA) and synthesis design that incorporated a novel drug release mechanism—specifically, core-shell nanospheres that reversibly capture an antibiotic suitable for treating bacteria-induced eye infections. The resulting hydrogels were transparent, were of appropriate size and shape, and provided for sustained drug release capable of inhibiting the proliferation of two important bacteria strains relevant to ocular infections, *S. aureus* and *P. aeruginosa*.

Ciprofloxacin was chosen as the antibiotic to be encapsulated in the nanospheres because of its broad use in the treatment of both Gram-positive and -negative bacteria, supported by our initial studies of growth inhibition by as little as 1.5 μg of ciprofloxacin per milliliter of cultures inoculated with either *S. aureus* or *P. aeruginosa* (Figs. 2A, 2B). Importantly, ciprofloxacin is well tolerated in humans, even with intravenous administration, and it is one of the most widely used antibiotics for ocular infections, including bacterial conjunctivitis or more severe infections that include bacterial keratitis.[50–52] Reports of resistance to ciprofloxacin have increased in recent years, which has prompted the development of newer ophthalmic fluoroquinolones, including

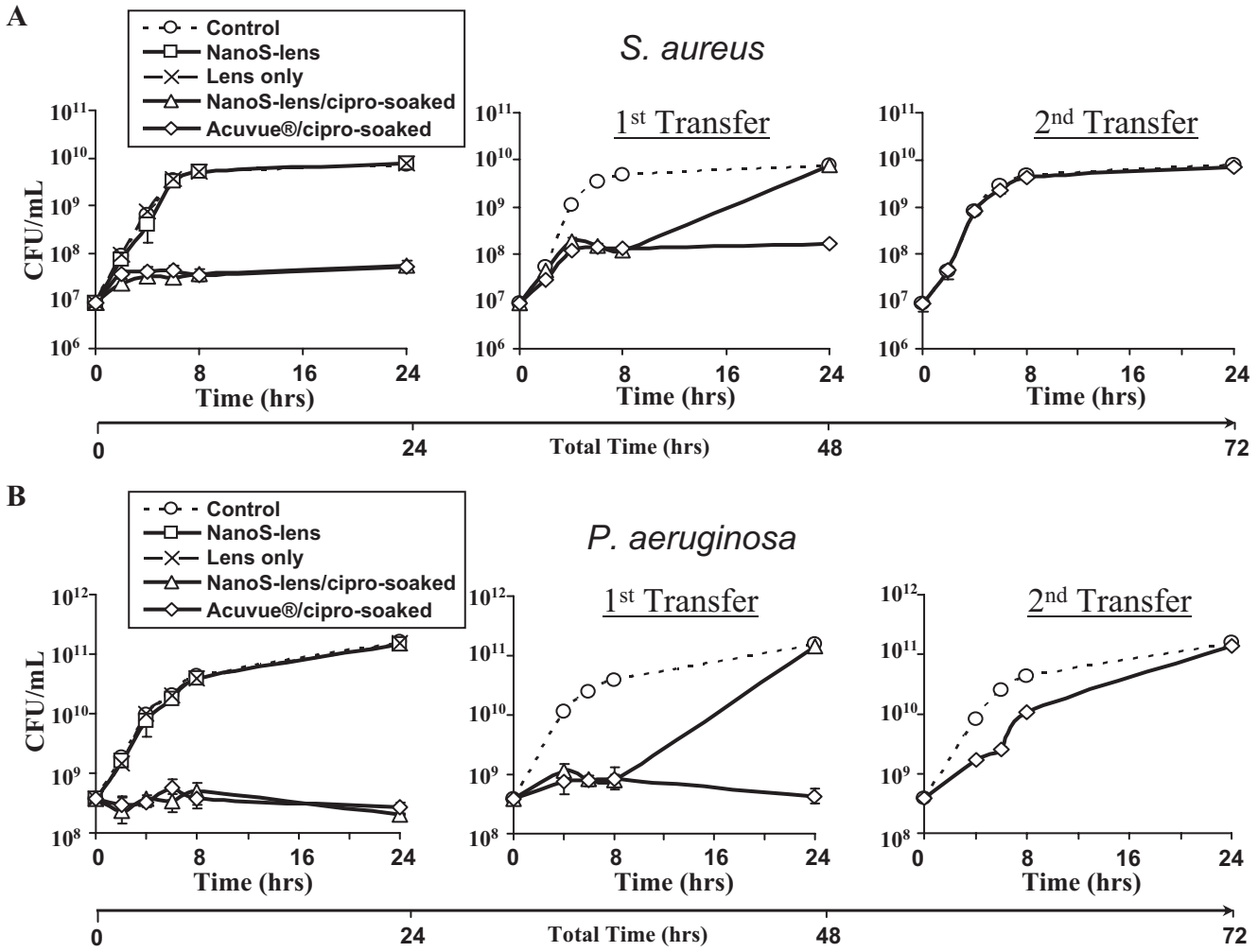

Figure 6. Drug-free nanospheres do not inhibit bacterial growth. Lenses were manufactured with drug-free nanospheres (NanoS-lens) or with no nanospheres (Lens only) and added directly to bacterial cultures of (**A**) *S. aureus* or (**B**) *P. aeruginosa*. Shown are the amounts (CFU/mL) corresponding to OD_{600} measurements that were performed over the first 24 hours (*left*) and then after the lenses were transferred twice to fresh medium inoculated with bacteria from overnight cultures (1st transfer and 2nd transfer, *middle* and *right*). In all sets, the drug-free lenses showed growth identical with that of bacteria alone (some results are obscured from view). Manufactured drug-free nanosphere-incorporating lenses and Acuvue lenses (Johnson & Johnson Vision Care, Inc.) were also soaked in ciprofloxacin for 4 hours, rinsed in PBS, and added to bacterial cultures. Time lines below each graph indicate total times of each experiment.

levofloxacin, gatifloxacin, and moxifloxacin.[53] Nonetheless, ciprofloxacin is still widely used worldwide for treating ocular infections and is readily available as both the free base and HCl salt forms. These two forms allowed the testing of antibacterial activity provided by ciprofloxacin-soaked contact lenses versus that provided by the hydrophobic form released from the nanospheres. Furthermore, the effective use of a hydrophobic drug for encapsulation implies that this technology is applicable to a wide range of hydrophobic drugs, including steroids (e.g., hydrocortisone, prednisolone, betamethasone, and progesterone), anticancer drugs (e.g., paclitaxel, amphotericin B, rapamycin, and cyclosporine) and β-blockers for glaucoma treatment (levobunolol and timolol).[54–56] It is reasonable to assume that other fluoroquinolones would function similarly to ciprofloxacin after nanosphere encapsulation, since the newer families of fluoroquinolones (e.g., third and fourth generation forms) use minor changes in their structure that are unlikely to affect interactions between the drugs and the nanospheres. In future studies, a broad range of different medications could be tested for encapsulation by the core-shell nanospheres and drug delivery rates and activities.

The design of the core-shell nanospheres was chosen to be compatible with effective delivery of hydrophobic drugs and for incorporation into a conventional contact lens. Pullulan is a hydrophilic, biodegradable polysaccharide that is synthesized by the fungus *Aureobasidium pullulans* and that has been proposed for use as a food texturizer.[57] It is particularly well suited for incorporation in a contact lens because it is transparent in the polymerized form, is nontoxic and nonimmunogenic, and has been successfully used as a drug carrier.[58] Polycaprolactone (PCL), a semicrystalline polyester composed of polymerized ε-caprolactone, is also biodegradable and has been extensively studied for its use as a component in copolymers designed for 3D scaffolds for tissue engineering applications or for drug delivery systems, including those that incorporate antibiotics such as ciprofloxacin.[59–62] Once bound to pullulan, an amphiphilic copolymer is formed that, when mixed in an aqueous solution, self assembles into micelles. By including the hydrophobic form of ciprofloxacin with the mixture, the drug became encapsulated within the self-associated PCL blocks that are surrounded by a shell of hydrophilic pullulan. The resulting pullulan-b-PCL nanospheres capture the internalized drug, but the permeability of PCL allows the low molecular weight of ciprofloxacin (~380 g/M) to escape in a slow but sustained fashion. Our previous studies have shown

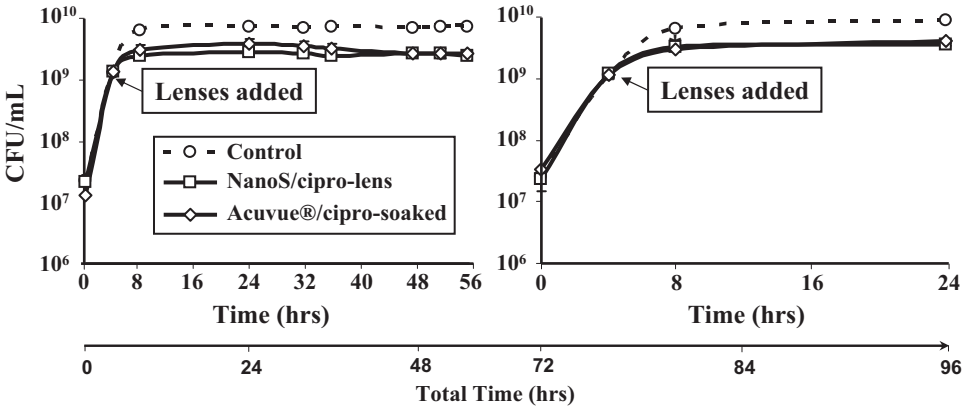

FIGURE 7. Inhibition of high doses of *S. aureus* by nanosphere/cipro-incorporating lenses. Bacteria from an overnight culture were used to inoculate broth, which was allowed to grow for 4 hours before the addition of nanosphere/cipro-incorporating lenses (NanoS/cipro-T-lens). Shown are the CFU/mL in medium with lenses as determined from OD_{600} measurements during the first 56 hours of growth before and after lens addition (*left*), and then colony-forming units/milliliter from inoculated broth with a new culture of bacteria before and after lens transfer (*right*). Data shown include measurements from inoculated broth alone (control) or broth with ciprofloxacin-soaked Acuvue (Johnson & Johnson Vision Care, Inc.) lenses (Acuvue/cipro-soaked), with a time line below the graphs indicating total time of the experiments. All differences observed between broth-only versus broth with added lenses at 8 hours or more after lens addition are significant ($P \leq 0.0001$, as determined by Student's *t*-test).

that inclusion of ciprofloxacin-HCl at ~1.8 mg/mL in the dialysis water not only prevented loss of encapsulated ciprofloxacin during dialysis but also increased the total amount of it within the final nanospheres.[46] Our analysis of the final pullulan-b-PCL nanospheres indicated that the antibacterial activity provided by encapsulated ciprofloxacin can be predicted. For example, we had shown that approximately 25% of the total weight of nanosphere/cipro is available ciprofloxacin, and ~75% of available drug is released over the first 24 hours. Release was continuous and steady over the first 8 hours (i.e., 2%–3% of the total encapsulated drug was released every hour).[46] Therefore, ~20% by weight of nanosphere/cipro is bioactive ciprofloxacin that will be released over the first 24 hours. The results shown here were consistent with these measurements: the addition of 7.5 to 10 μg/mL of nanosphere/cipro to bacterial-inoculated medium provided the same antibacterial activity as 1.5 to 2 μg/mL of ciprofloxacin-HCl (Figs. 2, 3). Thus, these pullulan-b-PCL nanospheres effectively encapsulated the antibiotic, maintained its biological activity, and provided for continuous antibacterial activity over 24 hours of culture in a predictable fashion.

The use of HEMA for the synthesis of the contact lenses that incorporated the nanosphere/cipro demonstrated that generation of this drug releasing system does not require any special lens synthesis procedures, and therefore is amenable to wide-scale production. HEMA is a well-characterized monomer for contact lens synthesis, and has been FDA-approved for ocular applications for many decades.[63] The synthesis procedure used here was based on well-established protocols, and nanosphere/cipro formed a clear solution that could be incorporated into lens synthesis. This method allowed for the generation of a transparent lens with typical characteristics of conventional contact lenses. Furthermore, the total amount of ciprofloxacin available to treat bacterial infections and the duration of its release could be adjusted by changing the design of the lens mold to create a thicker lens. For example, an average thin lens of 30 mg contained ~270 μg of available ciprofloxacin, most of which was released during the first 24 hours. By comparison, the thicker lens contained substantially more ciprofloxacin (1.2 mg), and this change did not appear to affect the overall characteristics of the lenses (i.e., the lenses retained clarity; see Fig. 4A). This thick lens also exhibited a slower rate of ciprofloxacin release: whereas the thin lens released ~93% of its calculated available drug over the first 24 hours, the thick lens released only 76% by 24 hours. This slower release combined with the increased total amount of drug incorporated provided for the longer antibacterial activity to both *S. aureus* (4 days) and *P. aeruginosa* (3 days). By comparison, contact lenses soaked in pharmaceutical grade ciprofloxacin developed precipitated drug on the lens that could not be removed by simply washing the lens in buffer (see Fig. 4C). Thus, the use of the pullulan b-PCL nanospheres is not only predicted to provide for more sustained drug release, but also allows incorporation of different quantities of drug in lenses that retain transparency.

Since the nanosphere-encapsulated ciprofloxacin release profile can be adjusted with the thickness of lens synthesized, lenses can be produced that provide for different treatment parameters (i.e., the duration of treatment or the total amount of bacteria that can be treated). This was reflected by the release studies and antibacterial activity assays of thin versus thick lenses. The thinner lens released over 250 μg of ciprofloxacin over the first 24 hours, which was more than adequate to inhibit bacterial growth of a typical eye infection of approximately 10^6 microbes,[64] as shown in Figure 5. This amount was also enough to inhibit the growth of greater than 10^9 bacteria in the high-titer assay (Fig. 7), in which the bacteria were allowed to expand in density before lens application. The amount released on day 2 was also enough to inhibit the growth of a secondary inoculation, but the amounts released thereafter were insufficient to effect a third inoculation. By comparison, the thicker lenses produced significant levels of ciprofloxacin for at least 3 days, which were sufficient to inhibit the growth of three separate inoculations of at least 10^7 microbes in the liquid assays (Fig. 8). Such lenses would be more appropriate for more severe cases of infection such as corneal bacterial ulcers where there are greater chances of vision loss without aggressive treatments. Both lenses would be sufficient to inhibit the growth of any residual bacteria that survived the first 24 hours of treatment, which would certainly be fewer than the number of bacteria used to inoculate the test cultures in our multiple transfer assays; either lens may also help to prevent resistant forms of bacteria from arising, as can occur when treatment is prematurely terminated. These results demonstrate the flexibility of the system and the potential of producing lenses with different amounts of incorporated drug applicable to different treatment regimens.

There remain several parameters of this contact lens-based drug delivery system that require further investigation. First, the lens drug release studies indicated that there may be batch-to-batch differences in the total amount of ciprofloxacin contained in the pullulan-b-PCL nanospheres; therefore, the synthesis procedures must be refined further. The need for improvement may also be applicable to the lens synthesis procedures, since our prototype thicker lens design may introduce variability in the actual amount of nanosphere/cipro that becomes incorporated in each lens. Second, the shelf life of the

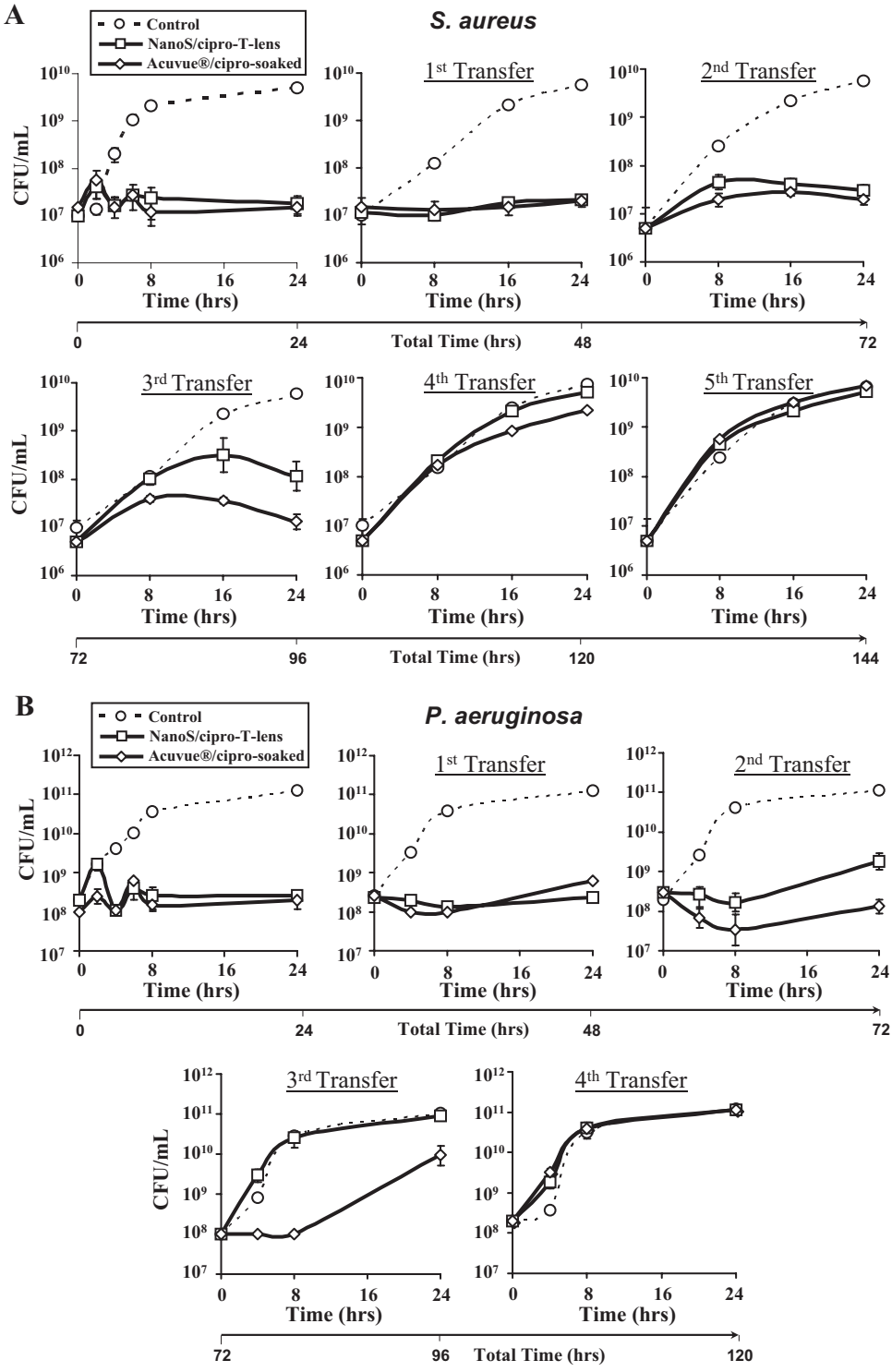

Figure 8. Antibacterial activity of nanosphere/cipro-incorporating thick lenses compared to ciprofloxacin-soaked Acuvue lenses (Johnson & Johnson Vision Care, Inc.). Both the thicker lenses with incorporated nanosphere/cipro (NanoS/cipro-T-lens) and the ciprofloxacin-soaked Acuvue lenses (Acuvue/cipro-soaked) were tested for extended growth inhibition (**A**) after five separate transfers to broth inoculated with live *S. aureus* or (**B**) after four separate transfers to broth inoculated with live *P. aeruginosa*. Below each sets of graphs are time lines indicating the total times of each experiment.

synthesized lenses was not tested, although the antibacterial activity assays were performed several weeks after the lenses were synthesized. The lens shown in Figure 4A was stored in a semihydrated (e.g., minimal amount of buffer) state before the antibacterial assays and retained its optical clarity after full hydration. The lenses may therefore be stored in minimally hydrated or even dehydrated states, which would significantly extend the shelf life of the captured ciprofloxacin. Finally, additional drugs could also be tested, including the newer, fourth-generation fluoroquinolones and therapeutic agents for other ocular disorders. Although these are goals for future studies, those presented here demonstrate that the pullulan b-PCL nanospheres are an attractive mechanism for encapsulating hydrophobic drugs and that the nanospheres can be incorporated into an aqueous-based delivery mechanism, promising a new approach to the design of ocular drug delivery systems.

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